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(54) Title: METHODS FOR INCREASING THE BIOAVAILABILITY OF BIOLOGICAL ACTIVE AGENTS			
(57) Abstract Methods for increasing the bioavailability of a potential nonpeptide medicinal agent by covalent attachment to a chemical moiety resulting in formation of a conjugate having increased bioavailability and water solubility relative to the unconjugated agent. Medicinal agents include biologically active flavonoid and nonflavonoid compounds whose water solubility and bioavailability is increased through covalent attachment to moieties that include inositol phosphate and bisphosphonate molecules.			

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METHODS FOR INCREASING THE BIOAVAILABILITY
OF BIOLOGICAL ACTIVE AGENTS

5 Background of the Invention

This invention relates to methods for enhancing the bioavailability of nonpeptide biologically active molecules.

10 Many potential medicinal agents are not therapeutically useful because of their poor bioavailability. A potential medicinal agent may exhibit desirable in vitro and in vivo biological activity, yet not be therapeutically useful because of low absorption, poor solubility, or inability to reach the appropriate
15 target site in a concentration sufficient for effectiveness. Poor bioavailability may result from one or more of such characteristics.

 Flavonoids are an example of compounds with desirable biological activities whose use as therapeutic
20 agents is limited by poor bioavailability. Flavonoids are flavone (2-phenylchrome; 2- phenyl- -benzopyrene; 2-phenyl-1, 4-benzopyron) derivatives constituting a class of naturally occurring molecules isolated from various plants. Some of the naturally occurring
25 flavonoids include apigenin (4', 5-7-trihydroxyflavone); genistein (4', 5, 7, trihydroxy-isoflavone); quercetin (3, 3', 4', 5-7 pentahydroxy-flavone); rutin (quercetin-3-rutinoside); conjugated forms such as quercetin pentaacetate, and quercetin 3-D-galactoside;
30 and phenolic and lipophilic polymethoxylated flavonoids, such as nobiletin and tangeretin (Kandaswami et. al (1991) Cancer Lett. 56:147-52). Examples of naturally occurring flavonoids are shown in Figure 1.

 In vitro studies in cell culture have demonstrated
35 that certain flavonoids have antiproliferative

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properties, and can inhibit the growth of cultured human malignant cells derived from primary human colon, gastric, ovarian, breast and squamous cell carcinomas, human osteosarcomas and leukemias. (Edwards et al. (1979) J. Nat. Prod. 42:85-91; Smolina et al. (1975) Cancer Res. 35:1865-1872; Molnar et al. (1981) Neoplasma 28:11-18; Catillo et al. (1989) supra; Hirano et. al (1989) Res. Commun. Chem. Pathol. Pharmacol. 64:69-78; Mori et al. (1988) Phytochemistry 27:1017-1020; Yoshida et al. (1990) FEBS Lett 260:10-13; Bracke et. al. (1989) Clin. Exp. Metastasis 7:283-300; Kandaswami et al. (1991) supra; Scambia et al. (1991) Cancer chemoth. and Pharmac. 28:255-258; Verma et al. (1988) Cancer Res. 48:5754; Hoffman et al (1989), Brit. J. Cancer 59:347; Larocca et. al. (1991), Brit, J. Haemat. 79:562; Yoshida et al., (1992), Cancer Res. 52:6676; Ranelletti et al. (1992), Int. J. Cancer 50:486).

In vivo studies in rats demonstrated that intraperitoneal injection of quercetin inhibited significantly the growth of squamous cell carcinomas in implantable cell growth chambers in rats (Castillo (1989) supra), and inhibited the phosphorylation activity of the Rous sarcoma virus transforming gene product in vitro and In vivo (Graziani et. al. (1983) Eur. J. Biochem. 135:583- 589). The activity of several enzymes, and the release of histamine from rat mast cells and human basophils, are affected by certain flavonoids (reviewed by Middleton (1984) TIPS 5:335).

The in vivo antoproliferative effects of flavonoids have been studied in mice treated with various tumor promoters. Quercetin was shown to reduce skin tumors initiated with 7,12- dimethylbenz (a) anthracene (DMBA) and promoted by teleocidin (Nashio et al. (1984) Gann 75:113- 116) or 12-O-tetradecanoylphorbol-13-acetate (TPA) (Nashio et al. (1984) Oncology 14:120-123).

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Similar results were obtained with apigenin which, like quercetin, exhibited antipromoter activity in mice treated with DMBA and promoted with TPA (Wei et al. (1990) Cancer Res. 50:499-502). The flavonoids
5 kaempferol and luteolin also blocked the increase in teleocidin-induced epidermal ornithine decarboxylase (ODC), although in that model quercetin was reported to have an insignificant effect (Fujiki (1986) Prog. Clin. Biol. Res. 213:429-440). Other flavonoids such as morin
10 (2', 3, 4', 5,7- pentahydroxyflavone), fisetin, and kaempferol reduced significantly the increase in epidermal ODC activity in mice induced by TPA treatment (Nakadate et al. (1984) Gann 74:214-222). Both quercetin and myricetin inhibited polycyclic aromatic hydrocarbon-
15 (PAH) and N-methyl-N- nitrosourea-induced skin tumorigenesis in mice (Mukhtar et al. (1988) Cancer Res. 48:2361-2365). Application of catechin, epicatechin, and their semisynthetic flavonoids catechin dialkyl ketals and epicatechin-4-alkylsulphides, inhibited TPA-induced
20 OCD activity in mouse epidermis in vivo (Gali et. al. (1993) Cancer Lett: 73:149-156).

The inhibitory potencies of several flavonoids on tyrosine protein kinases and serine/threonine kinases has been reported (Hagiwaze et. al. (1977) Biochem.
25 Pharmacol. 37:2987-2992; Graziani et al. (1983) supra; Akiyama et al. (1987) J. Biol. Chem. 262:5592-5595). The inhibitory effects of 41 synthetic flavonoid analogues on protein tyrosine kinase activity have been reported (Cusman et al. (1991) J. Med. Chem. 34:798-806).
30 Examples of active synthetic flavonoids are shown in Fig. 2.

Inhibition of cellular kinases may lead to "rapid apoptotic cell death" as demonstrated recently by Uckun et. al. (1995), Science 167: 886-891. These
35 investigators targeted the flavonoid genistein to B-cell

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precursor leukemic cells in mice. Targeting was achieved through the conjugation of genistein with a specific antibody that binds to the CD19 receptors which are on the surface of the leukemic cells. "More than 99.999 percent of human B-cell precursor leukemia cells were killed, which led to 100 percent long-term event-free survival (in mice) from an otherwise invariably fatal leukemia" -. As shown previously, the isoflavonoid genistein is a potent, and specific inhibitor of tryosine protein kinases (Akiyama et al. (1987) J. Biol. Chem. 262; 5592-5595. This in vivo study confirmed previous data in cell culture which demonstrated the inhibitory effects of other flavonoids such as quercetin, on the proliferation of human leukemic cells in culture, including human leukemic cell line K562 (Hoffman et al. (1988) Brit. J. Cancer 59: 347-348); acute myeloid and lymphoid leukemia progenitors (Larocca et al. (1991) Brit. J. Haematol. 79: 562-566); and human leukemic T-cells (Yoshida et al. (1992) Cancer Res. 52: 6676-6681). Anti-inflammatory, anti-allergic, anti-viral, and anti-hemorrhagic effects of flavonoids have been reported (Middleton (1984) supra).

The efficacy of both naturally occurring and synthetic flavonoid derivatives depends, in part, on the extent of their absorption, distribution, metabolism, and excretion (Middleton et al. (1984) supra). Flavonoids are relatively insoluble in water. Pharmokinetic studies in humans suggest that less than 1% of orally administered quercetin (4 grams) was absorbed unchanged (Gugler et al. (1975) Eur. J. Clin. Pharmacol. 9:229).

The therapeutic use of certain non-flavonoid compounds is also limited by poor bioavailability due to their low water solubility. An example is the alkaloid camptothecin

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(Wall et. al. (1966) J. Am. Chem. Soc. 88: 3858), which has been shown to have efficiency in animal tumor models. Camptothecin contains an intact hydroxyl residue at position 20 of the E ring which is essential for its antitumor activity (Wall et. al. (1977) Annu. Rev. Pharmacol. Toxicol. 17: 117; Hertz et al (1989) J. Med. Chem. 32: 715; Kingsbury et. al. (1991) J. Med. Chem. 34: 98-107; Hutchison (1981) 1065, review). As noted by Kingsbury et. al. (1991) 37: 1047 - *ibid*, "The poor water solubility of camptothecin precluded its development as a clinical agent and necessitated the use of the water-soluble sodium camptothecin". These investigators converted camptothecin into a water soluble form (1 mg/ml H₂O) by first modifying the molecule through the attachment of a hydroxyl group at position 9 or 10 (10-hydroxycamptothecin) and then conjugating covalently the added hydroxyl with a number of aminoalkyl groups. This conjugation did not utilize a linker element between the hydroxyl group at position 9 or 10 and the aminoalkyl compound, and it avoided the conjugation of the native hydroxyl group of camptothecin at position 20 (E ring).

Summary of the Invention

The invention features methods for enhancing the bioavailability of therapeutic compounds. A conjugate is formed by covalently attaching to the agent a chemical moiety which is highly bioavailable, resulting in increased bioavailability of the agent. As shown in our examples given below we were capable of conjugating the native, unmodified hydroxyl groups of therapeutic agents to moieties via a linker element, a process yielding molecules of high water solubility (~100mg/ml H₂O vs. 1mg/ml H₂O for the alkylated camptothecin), and retaining full biologic activity.

By the term "agent" or "potential medicinal agent" is meant a compound having desirable *in vitro* and/or *in*

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vivo biological activity in experimental animals which could potentially be used in the treatment of a disease or malignancy. In one embodiment of the invention, the agent is a flavonoid or flavonoid derivative. In another
5 embodiment, the agent is a nonflavonoid.

By the term "bioavailability" is meant the extent at which an agent is water soluble and therefore injectable, or capable of trespassing the intestinal wall so that it can be absorbed into a living system or is
10 made available at the site of physiological action. An increase in bioavailability means that a larger amount of the administered agent reaches a relevant target site.

By the term "chemical moiety" is meant a compound possessing high bioavailability and which confers
15 enhanced bioavailability to an agent as a conjugate. The chemical moiety to be covalently attached to the agent will vary depending on the specific agent used. In one embodiment of the invention, the chemical moiety increased bioavailability to an agent is inositol
20 phosphate. By the term "inositol phosphate" is meant inositol having one or more phosphate groups, including mono-phosphorylated inositol, di-phosphorylated inositol, or poly-phosphorylated inositol. The invention may also be practiced with other carbohydrates,
25 phosphorylated carbohydrates or other chemical groups, including phosphate group(s), sulfate group(s), carboxyl group(s), acyl groups(s), amino acids, peptides, proteins, glutathione, glyceraldehyde or derivatives, nucleoside, nucleotides, polyols, phosphonates, including
30 bisphosphonates, aminoalkyl molecules, and/or polyamines, or combinations of these groups.

By the term "conjugate" is meant a synthetic chimeric molecule formed by a covalent bond or bonds between a chemical moiety and a potential medicinal
35 agent, or between a chemical moiety, a linking element,

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and the agent molecule. In conjugate form, the potential medicinal agent possesses enhanced bioavailability relative to the unconjugated agent.

5 In one embodiment, the increased bioavailability of an agent results from an increase in its solubility in a biological fluid. Solubility of the agent in the desired medium results from modifying the polarity of the agent. Accordingly, in one aspect, the bioavailability of an agent is increased by covalent attachment of a
10 chemical moiety that alters the polarity of the agent, increasing its solubility in the desired medium.

A third method of increasing the bioavailability of an agent is to effectively increase the concentration of the agent at the target cell. Accordingly, in one
15 aspect, the bioavailability of an agent is increased by covalent binding to a chemical moiety which binds a specific target cell. The resulting conjugate binds the target cell, increasing the concentration of the agent at the target cell. In one embodiment, the chemical moiety
20 is actively transported into the target cell. The resulting conjugate is transported into the target cell, resulting in delivery of the bound agent to the target cell. An example of a chemical moiety actively transported into a target cell is inositol phosphate,
25 which is transported into cells having an active inositol phosphate transport system. Another example of chemical moieties that target to specific sites are the bisphosphonates which are taken up by the skeleton (Gertz et al. (1993) Osteoporosis International 3 suppl. 3: S
30 13-16; Fleisch (1991) Drugs 42: 919-944).

The conjugate may be formed by direct covalent attachment to a chemical moiety to the agent, or by indirect attachment to a chemical moiety via a linking element. By "linking element" is meant a chemical
35 element connecting the agent and chemical moiety.

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Preferably, the linking element is a carbon chain having between 1 to 5 carbons in length and having one or more reactive moieties such that other groups may be attached to the linking element. Depending on the length of the carbon chain and the groups attached, the linking element may increase the aqueous or lipid solubility of the agent. Thus, the linking element may function to enhance the bioavailability of the agent or may be utilized to facilitate conjugate synthesis. In a preferred embodiment, the linking element is succinate. In another embodiment, the linking element connects multiple agent molecules to a cell targeting chemical moiety, this increasing the effective concentration of the agent at the target site.

The invention encompasses various methods for attachment of the chemical moiety to the potential medicinal agent. In one embodiment of the invention, a conjugate is formed by attachment of inositol phosphate to a flavonoid molecule. In a specific embodiment, the conjugate is formed by attachment of inositol phosphate to a flavonoid through the phosphate group of the inositol (for example, see Fig. 3A). Alternatively, the molecules are coupled via a linking element through the phosphate linkage (for example, see Fig. 3B). In a third embodiment, the molecules are coupled via a linking element at a position on the inositol other than the phosphorylated position (for examples, see Figs. 3C and 3D). The invention features flavonoid-inositol phosphate conjugates where the conjugated flavonoid has enhanced bioavailability relative to the unconjugated flavonoid, resulting from improved water-solubility and/or cellular delivery. Inositol phosphate may be conjugated to flavonoids through a covalent bond or series of covalent bonds between inositol phosphate and one or more hydroxyl and/or amino residues of the

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flavonoid. The flavonoids useful in this invention may be naturally occurring or synthetic.

In another embodiment the therapeutic agent is covalently bound to a phosphonate, and preferably to a bisphosphonate, through a linking element. Said bisphosphonate may include, but is not limited to, alendronate, pamidronate, etidronate, clodromate, tiludronate, ibandronate or residronate (Figure 4). Additional examples of bisphosphonate structures are given in Shinoda et al. (1983) *Calcified Tissue International*, Springer Verlag, 35: 87-99. Bisphosphonates have been used in the treatment of several clinical conditions, namely ectopic calcification, ectopic bone formation, Paget's disease, osteoporosis and increased osteolysis of malignant origin (Dunn et al. (1994) *Drugs and Aging* 5: 446-474; Licata (1993) *Cleveland Clinic J. of Med.* 60: 284-290; Fitton and McTavish (1991) *Drugs* 41: 289-318; Green et al. (1994) *J. Bone and Mineral Res.* 9: 745-751; Gertz et al. (1993) 3: S13-16 - *ibid*; Fleisch (1994) *Seminars in Arthritic and Rheumatism* 23: 261- 2; Fleisch (1991) 42: 919-944 - *ibid*). Bisphosphonates may be conjugated to a flavonoid or to a nonpeptide nonflavonoid therapeutic agent having one or more hydroxyl groups and/or one or more amino groups. The bisphosphonates and the therapeutic compounds are coupled via a linking element at a position on the bisphosphonate other than the phosphorylated one(s).

Conjugation of therapeutic agents of low water solubility to bisphosphonates may increase their bioavailability by increasing both their water solubility and their localized delivery to bone. This is desirable in the treatment of osteosarcomas when the agent is cytotoxic for human malignant osteosarcoma cells. For example, apigenin, quercetin, and camptothecin exhibit

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strong antiproliferative effects on primary human osteosarcoma cells in culture. Agents conjugated to bisphosphonate which are capable of inhibiting tyrosine protein kinases, such as flavonoids (Hagiwaza et al. (1977) *ibid*; Grazian et al. (1983) *ibid*; Akiyama et al. (1987) *ibid*; Cushman et al. (1991) *ibid*; Uckun et al. (1995) *ibid*), may be delivered by the bisphosphonates to osteoporotic lesions in the bones of human patients with osteoporosis. Inhibition of tyrosine kinase activity in osteoclasts at the site of osteoporotic lesions may result in a decrease activity of bone resorption which is promoted by osteoclasts. Osteoclast proliferation has been shown to be sensitive to tyrosine kinase activity C. Hall et al. (1994) *Bioch. Bioph. Res. Commun.*, 199: 1237-1244; Yoneda et al. (1993) *J. Clin. Invest.*, 91: 2791-2795; Lowe et al. (1993) *Proc. Nat'l. Acad. Sci. USA*, 90: 4485-4489.

As described above, inhibition of tyrosine kinase activity on B-cell precursor leukemia cells by the flavonoid genistein resulted in the "rapid apoptotic cell death" of the leukemic cells in mice (Uckun et al. (1995) *ibid*). In that study the flavonoid genistein (which is a strong inhibitor to tyrosine kinase and is practically insoluble in water) was coupled to a specific antibody which targeted the flavonoid to the CD19-receptor of the leukemic cells. The methods of the invention may be used to increase the water solubility and site delivery of other non-flavonoid compounds. For example, the solubility of a compound in water may be increased by formation of a conjugate between a chemical moiety and a non-flavonoid compound having one or more hydroxyl groups and/or one or more amino groups.

The conjugate of the invention may be administered to a patient in a number of ways known to the art, including, intravenous, parenteral, intranasal, oral,

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topical, transdermal, and subcutaneous sustained release injectable implant formulations. Formulations may be prepared by any of the techniques known in the pharmaceutical arts. Such techniques are described, for example in Remington's Pharmaceutical Sciences ((1980) Mack Pub. Co., Easton, PA). Formulations for parenteral administration may contain common excipient such as sterile water or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes, and others of similar nature. In particular, biocompatible, biodegradable lactate polymers, lactate/glycoside copolymers, or polyoxethylene-polyoxy-propylene copolymers may be useful excipient to control the release of the conjugate of the invention. Other potentially useful parenteral delivery systems include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for administration by inhalation may contain excipient such as, for example, lactose. Inhalation formulas may be aqueous solutions containing excipient such as, for example, polyoxyethylene 9-lauryl ether, glycocholate and deoxycholate, or they may be oily solutions for administration in the form of nasal drops, or as a gel to be applied intranasal. Compositions for parenteral administration may also include glycocholate for buccal administration, or cistic acid for vaginal administration. Topical administration may utilize slow releasing patches.

The concentration of the conjugate in a physiologically acceptable formulation will vary depending on a number of factors, including the dosage to be administered, the route of administration and the condition being treated.

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Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Brief Description of Figures and Drawings

5 Fig. 1 provides examples of the chemical structures of a number of flavonoid compounds.

 Fig. 2 provides examples of the chemical structures of active synthetic flavonoids.

 Fig. 3 is a diagram illustrating four methods for
10 the synthetic coupling of inositol phosphate to a flavonoid derivative. Fig. 3A shows coupling of the components through the phosphate group. Fig. 3B shows coupling via a linking element through the phosphate linkage. Figs. 3C and 3D show coupling via a linking
15 element at a position on the inositol other than the phosphorylated one(s)..

 Fig. 4 contains examples of bisphosphonates.

 Fig. 5 and Fig. 6 are synthetic pathways for preparation of a phosphoinositol-succinate linked
20 quercetin.

 Fig. 7 is a synthetic pathway for preparation of a phosphoinositol-succinate linked apigenin.

 Fig. 8 is a synthetic pathway for preparation of a phosphoinositol-succinate linked genistein.

25 Fig. 9 is a synthetic pathway for preparation of an inositol phosphate linked regioselectively to quercetin via a glycerol linker.

 Fig. 10 is a synthetic pathway for preparation of a phosphoinositol quercetin linked through the phosphate
30 group.

 Fig. 11 Antiproliferative activity of quercetin and phosphoinositol-succinate linked quercetin on a primary human adenocarcinoma cell line (SW 480) in culture. The biologic activity of the water- soluble
35 conjugated quercetin (6c) is similar to that of

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unconjugated quercetin. In all biologic studies the unconjugated quercetin was dissolved in dimethylsulfoxide (DMSO).

5 Fig. 12 Antiproliferative activity of quercetin and phosphoinositol-succinate linked quercetin on a primary human osteosarcoma cell line (MG 63) in culture. The biologic activity of the water-soluble conjugated quercetin (6c) is similar to that of unconjugated quercetin.

10 Fig.13 A bisphosphonate-coupled flavonoid.

Detailed Description

Generally, the invention features methods for increasing the bioavailability of a potential medicinal agent such that the agent becomes therapeutically useful.
15 The methods of the invention are particularly useful with a biologically active agent whose therapeutic use is limited by low bioavailability. However, the invention may be used with any agent for which increased bioavailability is desired.

20 The bioavailability of any agent may be low due to a variety of factors, including low solubility and/or low rate of transport to its target cell. In some cases, the bioavailability of an agent may be low due to its inability to reach an effective concentration at the site
25 of the target cell. The invention provides methods for overcoming these problems, thus increasing the medicinal usefulness of a variety of biologically active agents.

For example, flavonoid derivatives have antiproliferative effects on malignant cells in vitro
30 and in experimental animals in vivo. Their therapeutic use is precluded, however, by their poor water solubility. The present invention provides for increasing the water solubility of the therapeutic agents. The methods of the invention may also be used to
35 increase the cellular delivery of any agent by allowing

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the agent to be actively transported into cells by the cell membrane inositol phosphate transport system. (See, Saltiel and Sorbara-Cazan (1987) Biochem. Biophys. Res. Commun. 149:1084-1092). Bisphosphonate-linked flavonoid
5 and nonflavonoid therapeutic compounds may be delivered to bone lesions of patients with osteosarcomas or osteoporosis.

The methods of the invention are preferably used with agents having biological activates which would be
10 useful in the treatment of diseases, such as the antiiproliferative effect of flavonoid in tumors, leukemia, and nonmalignant proliferative disorders such as psoriasis. By the term "flavonoid" is meant any flavone molecule containing one or more hydroxyl groups
15 and/or one or more amino groups.

Examples presented in this application demonstrate that conjunction of flavonoids in inositol phosphate via a linking element (Figures 6-9) resulted in a dramatic
20 increase of their water solubility, retaining their biologic activity. In these examples, apigenin, quercetin, and genistein, which are practically insoluble in water, were converted into highly soluble conjugates with water solubility of over 100 mg per milliliter at room temperature.

The examples provided herein illustrate formation of a conjugate by covalent bonding of inositol phosphate to a flavonoid. The covalent bond may be formed in a
25 number of ways, including coupling through the phosphate group (for example, see Fig 3A), coupling via a linking element through the phosphate linkage (for example, see
30 Fig 3B), and coupling via a linking element at a position on the inositol other than the phosphorylated one(s) (for examples, see Figs. 3C and 3D). Each synthetic method of the invention may be varied in the position(s)
35 of attachment to the inositol and the flavonoid, in the

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number and position of phosphate groups, in the length and chemical nature of the linking unit, in the flavonoid derivative coupled, and in the inositol isomer used (e.g., myo- or chiro-, etc.). Linking elements include succinate diester, 4-hydroxybutyrate ester phosphate diester, and glycerol diester. In one preferred embodiment, flavonoids are conjugated to inositol-2 phosphate via a succinate linker. Other suitable linkers include alkyl chains, mono or poly alcohol units, mono- or polyether units, thioethers, mono- or polyester units, mono- or polyamine units, mono- or polysulfates, mono- or polyarenes, mono- or polyphosphates, mono- or polyamides, peptides, mono- or polysulfides, mono- or polysaccharide, or a combination of these structures.

The invention is shown herein in what is considered to be the most practical and preferred embodiments. It is recognized, however, that departures may be made therefrom which are within the scope of the invention and that obvious modifications will occur to one skilled in the art upon reading this disclosure. The examples provided below describe the direct and indirect attachment of inositol phosphate to flavonoids and flavonoid derivatives. These examples are for illustration purposes only, and are not intended to limit the scope of the invention.

Example 1. Preparation of (quercetin-5-yl) (myo-inositol-2-phosphate-1-yl) succinate.

Compound 6c (quercetin-5-yl) (myo-inositol-2-phosphate-1-yl) succinate) may be prepared as illustrated in Figs. 5 and 6. 3,4,5,6-Tetra-benzyl-1-(1,1,1-trichloroethylchloroformyl)-Myo inositol. To 772.7 mg of compound 5a (1.43 mmol) (co-evaporated with toluene) in 4.0 ml of pyridine was added 197 EI (1.43 mmol)

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1,1,1-trichloroethylchloroformate at 0-C. The reaction was stirred at 0-C for 1 h. The reaction mixture was diluted with 2 ml of dichloromethane. The organic phase was washed with water (3 x 2 ml), dried, and evaporated. The crude products were co-evaporated with heptane until pyridine was no longer detected by smell and their chromatographed on silica gel with hexane: ether (3:1) (Rf=0.15) providing product as an oil with a 79% yield. 1H NMR (CDCl3) δ 2.35 (s, 1H, OH), 3.51- 3.57 (m, 2H, H3, H6), 3.97 (yt, J=9.5 Hz, 1H, H4, or H5), 4.14 (yt, J=9.8 Hz, 1H H4 or H5), 4.34-4.39 (m, 1H, H2), 4.61-4.79 (m, 6H), 4.84-4.91 (m, 5H), 7.24-7.38 (m).

3,4,5,6-Tetra-benzyl-2-dibenzylphosphoryl-1-(1,1,1-trichloroethylchloroformyl)-myo inositol (5b). To 414 mg of the product from above (0.578 mmol) (co-evaporated with toluene) and 122 mg of 1H-tetrazole (1.74 mmol) was added 2.5 ml acetonitrile and 578 μ l of N,N-diethyl dibenzylphosphoramidite (1.74 mmol). The reaction was stirred at room temperature for 20 min. The reaction mixture was then cooled to -42-C (MeCN/CO2) and 2.1 ml of 1 M 85% MCPBA in CH2Cl2 was added. The ice bath was immediately removed and the reaction mixture allowed to warm to room temperature. After ten minutes, 5 ml of 0.1 M NH4 HCO3 buffer (Ph 7.87) and 5 ml of 10% Na2S2O4 were added. The crude reaction mixture was extracted with CH2Cl2. The organic layer was washed with water (1 x 5 ml), dried, and evaporated. The crude products were chromatographed on silica gel with benzene:ethyl acetate (19:1) (Rf.33) providing 5b as an oil in a 62% yield. 31P NMR (CDCl3) δ 1.41 1H NMR (CDCl3) δ 3.54-3.63 (m, 2H, H6, H3), 3.90 (yt, J=9.5 Hz, 1H, H4, or H5), 3.97 (yt, J=9.6 Hz, 1H, H4 or H5), 4.59 (d, J=10.9 Hz, 1H, 1/2 CH2Ph), 4.63 (d, J=9.8 Hz, 1H, 1/2 CH2Ph), 4.67 (d, J=10.9 Hz, 1H, 1/2 CH2Ph), 4.72-4.92 (m, 9H), 4.96-5.00

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(m, 2H, CH₂Ph), 5.12- 5.16 (m, 2H, CH₂Ph), 5.37 (d, J=2.3, 8.6 Hz, 1H, H₂), 7.12-7.43 (m).

3,4,5,6-Tetra-benzyl-2-dibenzylphosphoryl-myo inositol. To 664 mg of 5b (0.669 mmol) was added to 10 ml THF/HOAc/H₂O (5:1:1) and 1.108 g of zinc dust was added. The reaction was stirred at room temperature for 1h. An additional 1.108 g of zinc dust was added and the reaction was stirred at room temperature for 1h. The reaction mixture was filtered through Celite, diluted with CH₂Cl₂. The organic layer was washed with water (1 x 5 ml), dried, and evaporated. The crude products were chromatographed on silica gel with benzene:ethyl acetate (8:1) (R_f=0.009) providing product as an oil in a 73% yield. ³¹P NMR (CDCl₃) I 0.95 1H NMR (CDCl₃) I 2.86 (d, J=5.0 Hz, 1H, OH), 3.47-3.53 (m, 2H, H₃, H₆), 3.54-3.59 (m, 1H, H₁), 3.73, (yt, J=9.4Hz, 1H, H₄, H₅), 4.55 (d, J=11.1 Hz, 1H, 1/2 CH₂Ph), 4.71 (d, J=11.2 Hz, 1H, 1/2 CH₂Ph) 4.73 (d, J=10.7 Hz, 1H, 1/2 CH₂Ph), 4.80-5.12 (m, 10H), 7.16-7.39 (m).

3,4,5,6-Tetra-benzyl-2-dibenzylphosphoroyl-1-succinyl-myo inositol (5c). To 200 mg of the product from above (0.234 mmol) (coevaporated with toluene) (was added 122.6 mg off succinic anhydride (1.23 mmol), 150.3 mg of 4-dimethylamionopyride (1.23 mmol), and 5 ml 1,2-dichloroethane. The reaction was stirred at room temperature for 2h. The reaction mixture was cooled to 0-C and 12 ml of 0.5 M HSO₄/SO₄ buffer (pH = 1.0) was added. The crude mixture was stirred for 5 min at 0-C. The mixture was extracted with CH₂Cl₂. The organic layer was washed with water (3 x 5 ml), dried, and evaporated. The crude products were chromatographed on silica gel with benzene:ethyl acetate (5:2) (R_f=0.05) providing 5c as an oil in a 82% crude yield. ³¹P NMR (CDCl₃) I 2.13. 1H NMR (CDCl₃) I 2.37- 2.64 (m, succinate and impurity), 3.53-3.61 (m, 2H, H₃, H₆), 3.88 (yt, J=9.5 Hz, 1H, H₄ or

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H5), 3.93 (yt. $J=9.7$ Hz, 1H, H4 or H5), 4.58 (d, $J=10.9$ Hz, 1H, 1/2 CH₂Ph), 4.63 (d, $J=11.3$ Hz, 1H, 1/2 CH₂Ph), 4.72-4.96 (m, 9H), 5.05 (m, 2H, CH₂Ph), 5.20 (yt, $J=2.1$, 8.5 Hz, 1H, H1), 7.08-7.15 (m, 2H), 7.18-7.38 (mm).

5 (3,6,3', 4')-Tetra-benzylquercetin-5-yl)

(3,4,5,6-tetrabenzyl-myo-inositol-2-phosphate-1-yl) succinate (6b). To 88.2 mg of crude 5c (0.0963 mmol) was added 6 ml of benzene and 200 μ l of oxalyl chloride. The reaction was stirred at room temperature for 2.5 h. The reaction mixture was anhydrously evaporated then dissolved in 2 ml 1,2-dichloroethane. To the reaction mixture was added 128 mg of 3', 4', 3,

7-tetra-benzylquercetin (0.193 mmol) (coevaporated with toluene) and 23.6 mg of 4-dimethylaminopyridine (0.193 mmol) in 1.5 ml 1,2-dichloroethane. The reaction was stirred for 5 min. The mixture was extracted with CH₂Cl₂. The organic layer was washed with water (3 x 1.5 ml), dried, and evaporated. The crude products were chromatographed on silica gel with benzene:ethyl acetate (8:1) ($R_f = 0.22$) providing 6b as an oil in a 63% yield. ³¹P NMR δ 1.51. ¹H NMR (CDCl₃) δ 2.38-2.80 (m, 2H, succinate), 2.99-3.17 (m succinate), 3.52-3.61 (m, 2H, H3 and H6 inositol), 3.91 (yt, $J=9.5$ Hz, 1H, H4 or H5), 3.96 (yt, $J=9.5$ Hz, 1H, H4 or H5), 4.55 (d, $J=10.9$ Hz, 1H, 1/2 CH₂Ph), 4.66 (d, $J=11.2$ Hz, 1H, 1/2 CH₂Ph), 4.70 (d, $J=11.9$ Hz, 1H, 1/2 CH₂Ph), 4.74 (d, $J=11.0$ Hz, 1H, 1/2 CH₂Ph) 4.81-4.86 (m, 7H), 5.00-5.06 (m, 2H, CH₂Ph), 5.13 (s, 2H, CH₂Ph), 5.18-5.25 (m, 3H), 6.71 (d, $J=2.6$ Hz, 1H, H6 or H8 quercetin), 6.82 (d, $J=2.4$ Hz, 1H, H6 or H8 quercetin), 6.93 (d, $J=8.7$ Hz, 1H, H5' quercetin) 7.10-7.49 (m), 7.64 (d, $J=.1$ Hz, 1H, H2' quercetin).

Quercetin-5-yl (myo inositol-2-phosphate-1-yl) succinate (6c). To 47.3 mg of 6b (0.030 mmol) was added 2 ml of ethanol/acetone (1:1) and 0.5 mg of 10% Pd/C. The reaction mixture was placed into a Parr apparatus under

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36 psi of hydrogen for 11 h. The reaction mixture was filtered through Celite and the filtrate washed consecutively with 3 ml each of acetone, acetone/water (1:1), and water. The collected solvent was evaporated providing 6c. This product is found to be extensively soluble in water) (solubility exceeds 10 g per 100 ml) UV (compound 6c in water): 357, 264, 205 nm.

Example 2.

(Apigenin-5-yl)-(myo-inositol-2-phosphate-1-yl)-succinate (7g).

An inositol phosphate linked to apigenin via a succinate linker may be prepared according to Figure 7. Briefly, p-methoxybenzylated diol 7a may be elaborated by a method analogous to that used in Example 1, to produce the acid chloride 7d. p-Methoxybenzylation of apigenin produces flavonoid 7e, which may be coupled with 7a in analogy to Example 1, and finally deprotected oxidatively to produce the title compound 7g.

Example 3.

(Genestein-5-yl)-(myo-inositol-2-phosphate-1-yl)-succinate (8c).

An inositol phosphate linked to genestein via a succinate linker may be prepared according to Figure 8. p-Methoxybenzylation of genestein may produce flavonoid 8a, which may be coupled with 7d in analogy to Examples 1 and 2, and finally deprotected oxidatively to produce the title compound 8c.

Example 4.

Inositol-1-yl-quercetin-5-yl-phosphate.

An inositol-phosphate-quercetin derivatives may be prepared as shown in Fig. 10. Generally, 3,4,5,6-tetrabenzyl myo-inositol is treated with benzyl bis(disopropylamino-phosphoramidite followed by a peracid

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to produce the 1,2-cyclic phosphate. Subsequently, the cyclic phosphate is coupled with quercetin (or a protected quercetin), which after catalytic hydrogenation produces the desired compounds.

5

Example 5.

1-(myo-inositol-2-phosphate-1-y)-2-(quercetin-5yl) glycerol.

Fig. 9 illustrates a synthetic pathway to a coupled quercetin with a glycerol linker. Generally, 3,4,5,6- tetrabenzyl myo-inositol is treated with allyl bromide, then phosphorylated. The peracid generates concomitantly an epoxide at the allyl group. Subsequent treatment with a protected quercetin, followed by deprotection provides the product.

Biologic Activity of Water-Soluble Conjugated Flavonoids. Biologic activity was determined in vitro in cultures of a primary human colon adenocarcinoma cell line (SW 480), and a human osteosarcoma cell line (MG-63). The malignant cells were plated in 24-well culture plates, and their population was measured after 6 or 9 days of culture in the presence or absence of the test material. In these studies, the unconjugated flavonoid was dissolved in dimethylsulfoxide (DMSO) and then diluted to the desired concentration with the culture media. The water-soluble conjugated flavonoid was dissolved in a minimum volume of distilled water and diluted to the desired concentration with the culture media. During the culture period the media of the cultured cells, with or without the test material, were changed every two days, and at the end of the culture period the cell population was counted. As seen in the examples shown in Figures 11 and 12, the antiproliferative activity of the phosphoinositol-succinate linked quercetin (hydroxyl position 5) was similar to that of

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the unconjugated quercetin in both the colon
adenocarcinoma (Fig. 11) and osteosarcoma (Fig. 12)
cultures.

5

What is claimed is:

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Claims

1. A method for increasing the bioavailability of a non-peptide therapeutic compound, said method comprising reacting said compound with a phosphate-containing chemical moiety to form a chemical bond between said compound and said moiety whereby bioavailability of said compound is increased.
2. A method for increasing the bioavailability of a non-peptide therapeutic compound, said method comprising reacting said compound at a native amino or hydroxyl group with a chemical moiety to form a chemical bond between said compound and said moiety whereby bioavailability of said compound is increased.
3. The method of claims 1 and 2, wherein said chemical moiety that contributes to increased bioavailability is inositol, an inositol phosphate, a carbohydrate, or a carbohydrate phosphate.
4. The method of claim 3 wherein said inositol phosphate is myo-inositol phosphate, L-myo-inositol phosphate, D-myo-inositol phosphate, L-chiro-inositol phosphate, or D-chiro-inositol phosphate.
5. The method of claim 3, wherein said inositol phosphate is selected from the group consisting of mono-phosphorylated inositol, diphosphorylated inositol, poly-phosphorylated inositol, and cyclic phosphates of inositol.
6. The method of claims 1 and 2, wherein said chemical moiety contains a sulfate group, a carboxyl group, an acyl group, a carbamate group, an amino acid, a

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peptide, a protein, glutathione, glyceraldehyde or derivatives, a nucleoside, a nucleotide, a polyol, an amino alkyl molecule, and/or a polyamine.

5 7. The method of claim 6 wherein said chemical moiety contains more than one of said groups or a combination of said groups.

10 8. The method of claims 1 and 2 wherein said chemical moiety is a phosphonate or bisphosphonate.

15 9. The method of claim 8 wherein said bisphosphonate is alendronate, etidronate, clodronate, pamidronate, tiludronate, residronate, or ibandronate.

20 10. The method of claim 1 and 2, wherein said chemical moiety is attached indirectly to the therapeutic compound via a linking element which links covalently the therapeutic compound and the chemical moiety, having the following general structure:



where TC is a therapeutic compound L is a linking compound, and M is the chemical moiety.

25 11. The method of claim 10, wherein said linking compound is a succinate diester, a 4-hydroxybutyrate ester, a phosphate diester, a glycerol diester, an alkyl chain, a mono or poly alcohol unit, a mono or polyether unit, a mono or polyester unit, a mono or polyamine unit, a mono or poly sulfate, a mono or polyarene, a mono or poly phosphate, a mono or polyamide, a peptide, a mono or polysulfide, a mono or polysaccharide, or a combination of these structures.

30

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12. The method of claim 10, wherein said linking compound connects a plurality of said therapeutic compounds to each chemical moiety.

5 13. The method of claims 1 and 2, wherein said chemical moiety binds covalently directly, or through a linker compound, to one or more hydroxyl and/or amino groups of the therapeutic compound.

10 14. The method of claim 1 or claim 2, wherein said therapeutic compound is a flavonoid or isoflavonoid, containing one or more hydroxyl or amino residues.

15 15. The method of claim 1 or claim 2, wherein said therapeutic compound is a nonflavonoid compound containing one or more hydroxyl or amino residues.

20 16. The method of claim 10 or claim 13, wherein said therapeutic compound is a nonflavonoid compound which is conjugated covalently to a linking compound through a native hydroxyl and/or amino residue, the linking compound also being bound covalently to said
25 moiety.

 17. A method for increasing the solubility of a therapeutic compound which contains one or more hydroxyl or amino groups, said method comprising reacting
30 said therapeutic compound with a solubility-increasing moiety to bond covalently said therapeutic compound to said moiety directly or through a linking compound via said hydroxy or amino residues of the therapeutic compound.

35

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18. The method of claim 17, wherein said increased solubility is increased aqueous solubility.

5 19. The method of claim 17 wherein said chemical moiety comprises one or more inositol phosphate groups.

10 20. The method of claim 19, wherein said conjugate is formed by covalent reaction of said inositol phosphate directly or through a linking compound with one or more hydroxyl groups of the flavonoid or nonflavonoid compounds.

15 21. The method of claim 19, wherein said conjugate is formed by covalent reaction of said inositol phosphate directly or through a linking compound with one or more amino groups of the flavonoid or nonflavonoid compounds.

20 22. A method for increasing the solubility of a non-peptide therapeutic compound which contains one or more hydroxy groups or amino groups, said method comprising reacting said agent with an inositol phosphate to bond covalently said agent to said inositol phosphate
25 directly or through a linking compound via said hydroxy and or amino groups of the therapeutic compound.

30 23. The method of claim 22, wherein said increased solubility is increased aqueous solubility.

35 24. A method for increasing the cellular delivery of a biologically active non-peptide agent which has one or more hydroxyl groups or amino groups, said method comprising reacting said agent with an inositol phosphate to bond covalently said therapeutic agent to

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said inositol phosphate directly or through a linking compound via said hydroxy or amino group to form a conjugate, which is transported into cells by a transport system for inositol phosphate or inositol phosphate-containing molecules.

25. The method of claims 22 and 24, where in said conjugate said inositol phosphate is substituted by at least one other non-inositol carbohydrate phosphate molecule.

26. The method of claims 10, and 13 , wherein said therapeutic compound is a flavonoid or a nonflavonoid, and said chemical moiety comprises one or more bishosphonate groups of claims 8 or 9.

27. A conjugate formed by covalent reaction of a bishosphonate directly or through a linking compound with one or more hydroxyl groups of a flavonoid or non-flavonoid therapeutic non-peptide compound.

28. The conjugate of claim 27 wherein said conjugate is formed by covalent reaction of said bisphosphonate directly or through a linking compound with one or more amino groups of the flavonoid or non-flavonoid compound.

29. A method for increasing the solubility of a non-peptide therapeutic compound which contains one or more hydroxy groups or amino groups, said method comprising reacting said agent with a bisphosphonate directly or through a linking compound via said hydroxy or amino groups.

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30. The method of claim 29, wherein said increased solubility is increased aqueous solubility.

5 31. A method for increasing the localized delivery to bone of a non-peptide therapeutic agent which has one or more hydroxyl groups or amino groups, said method comprising reacting said therapeutic agent with a bisphosphonate to bond covalently said therapeutic agent to said bisphosphonate directly or through a linking
10 compound via said hydroxy or amino groups to form a conjugate which is targeted to bone by the bisphosphonate groups of the conjugate.

15 32. The method of claim 31 in which said phosphate-containing moiety is linked to a native hydroxyl or amino group of said therapeutic compound.

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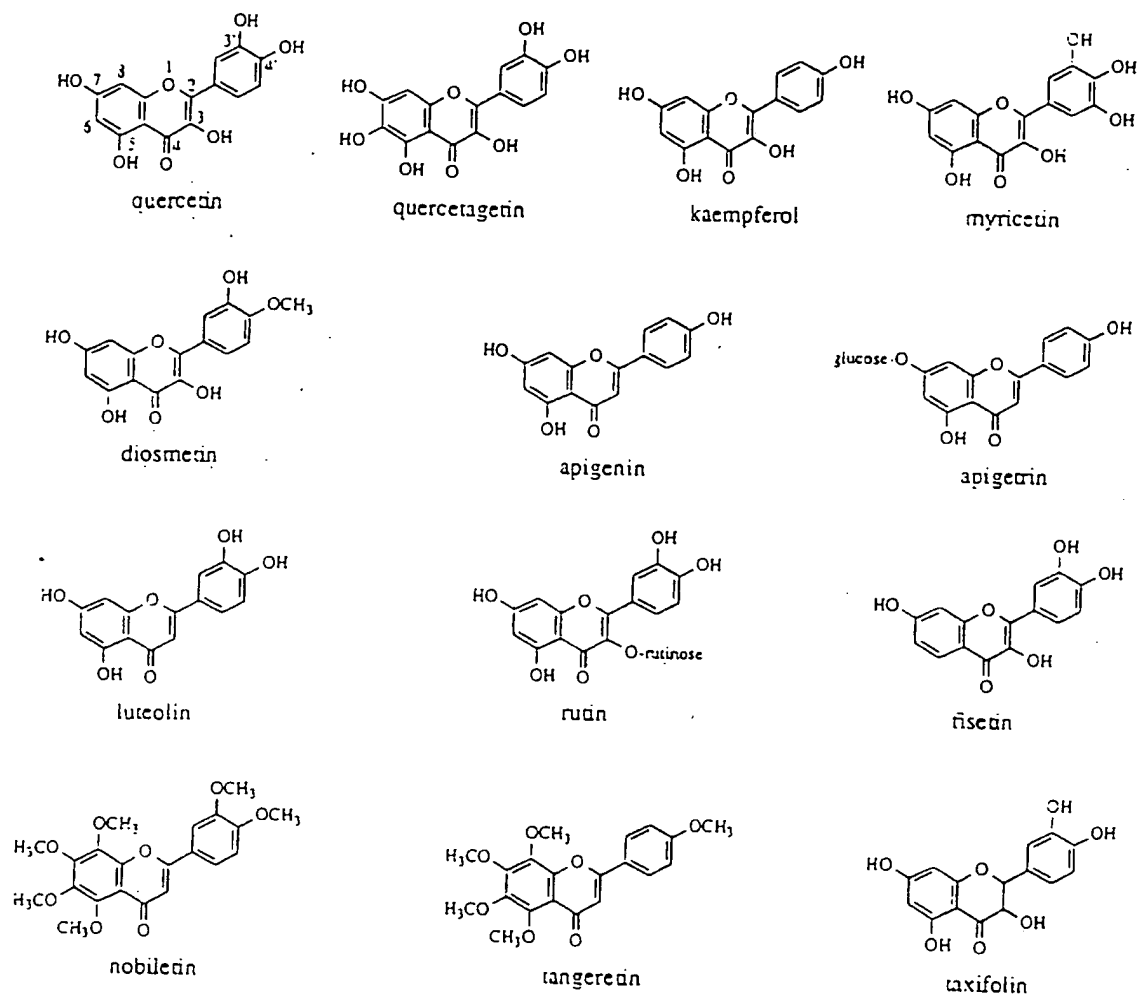
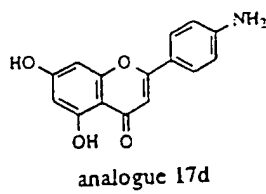
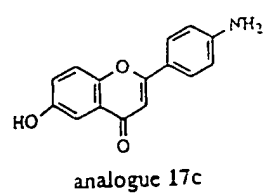
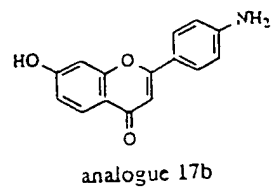
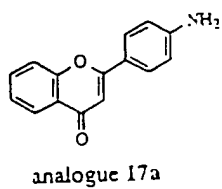
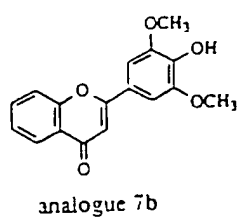
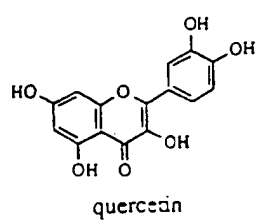


FIGURE 1

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(From Cushman, M., et al. *J. Med Chem.* 1991, 34, 798-806

FIGURE 2

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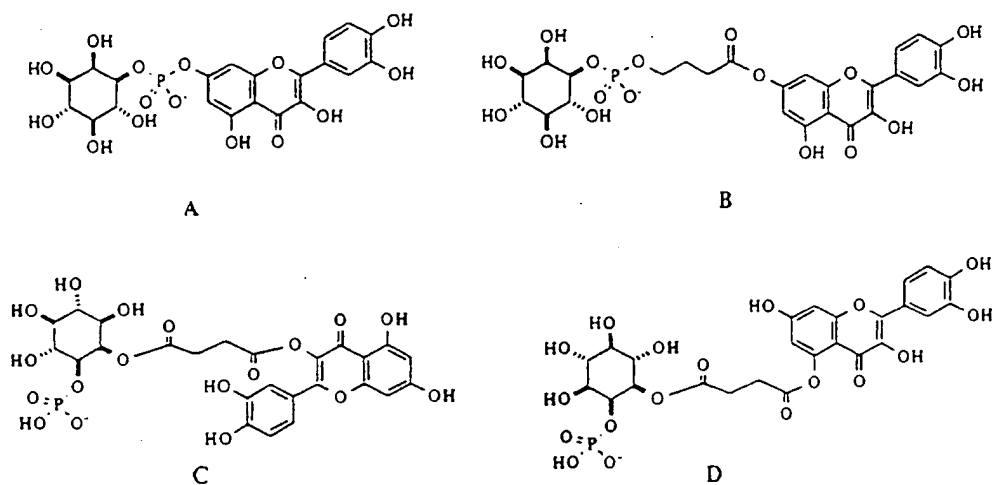
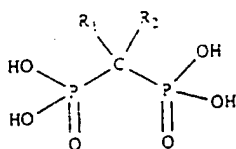


FIGURE 3

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name	R ₁	R ₂
etidronate	OH	CH ₃
alendronate	OH	CH ₂ CH ₂ CH ₂ NH ₂
pamidronate	OH	CH ₂ CH ₂ NH ₂
chlodronate	Cl	Cl

Examples of Bisphosphonates

FIGURE 4

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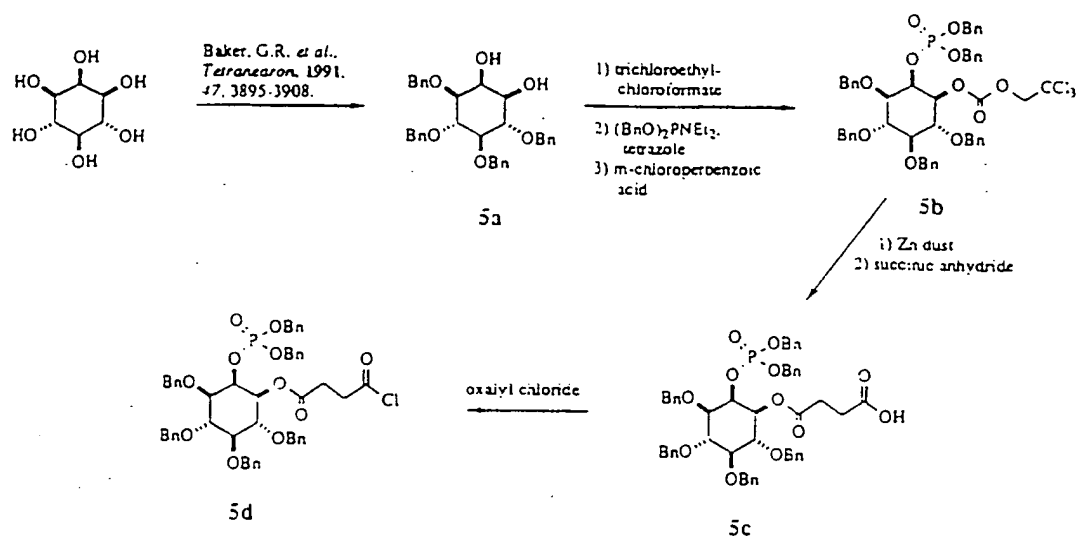


FIGURE 5

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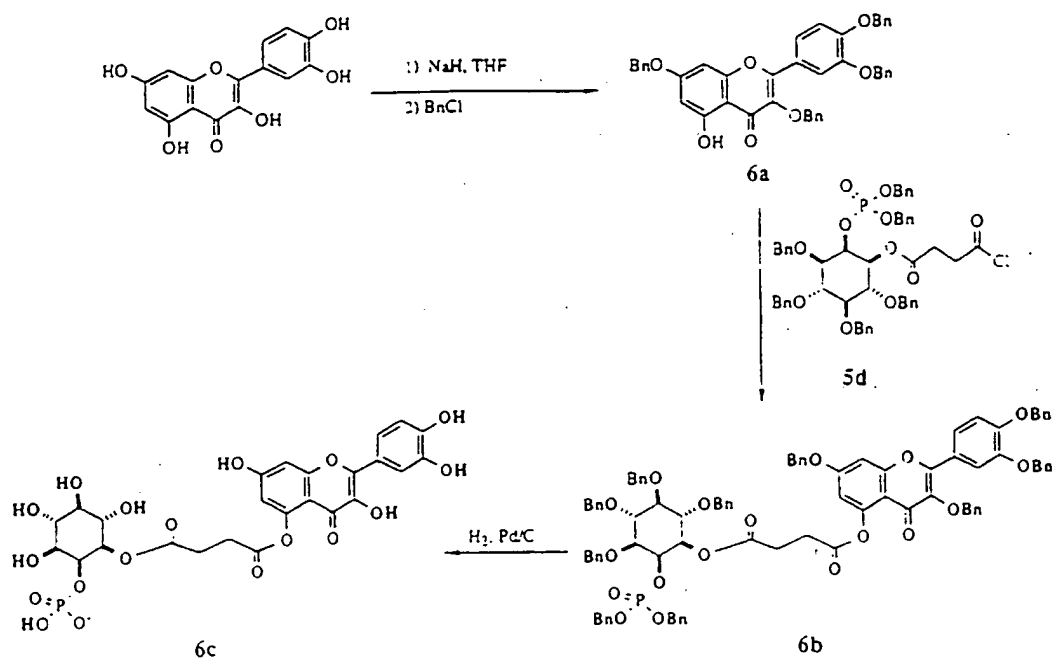


FIGURE 6

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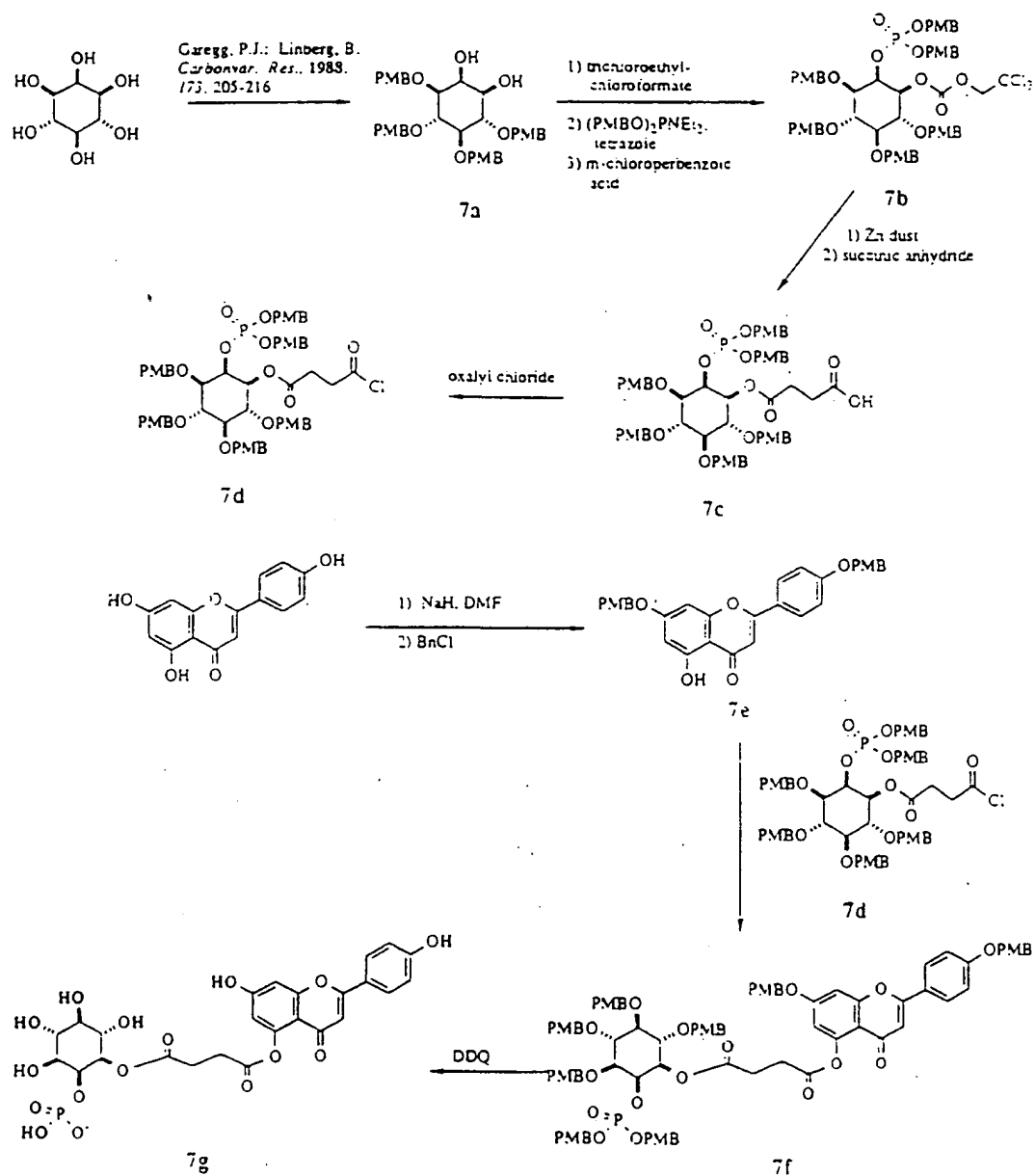


FIGURE 7

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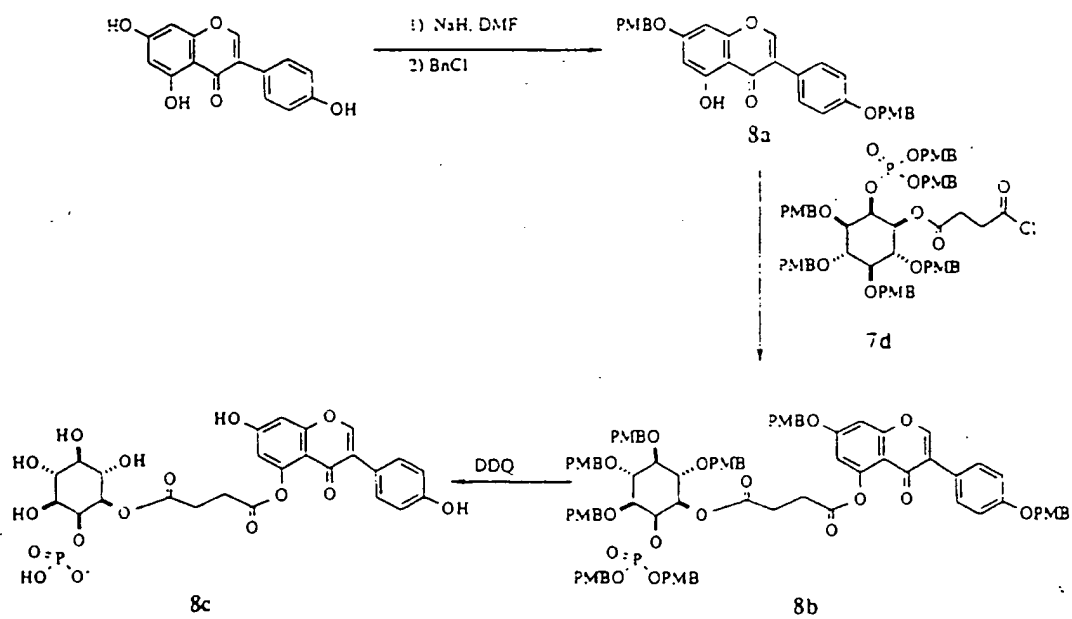


FIGURE 8

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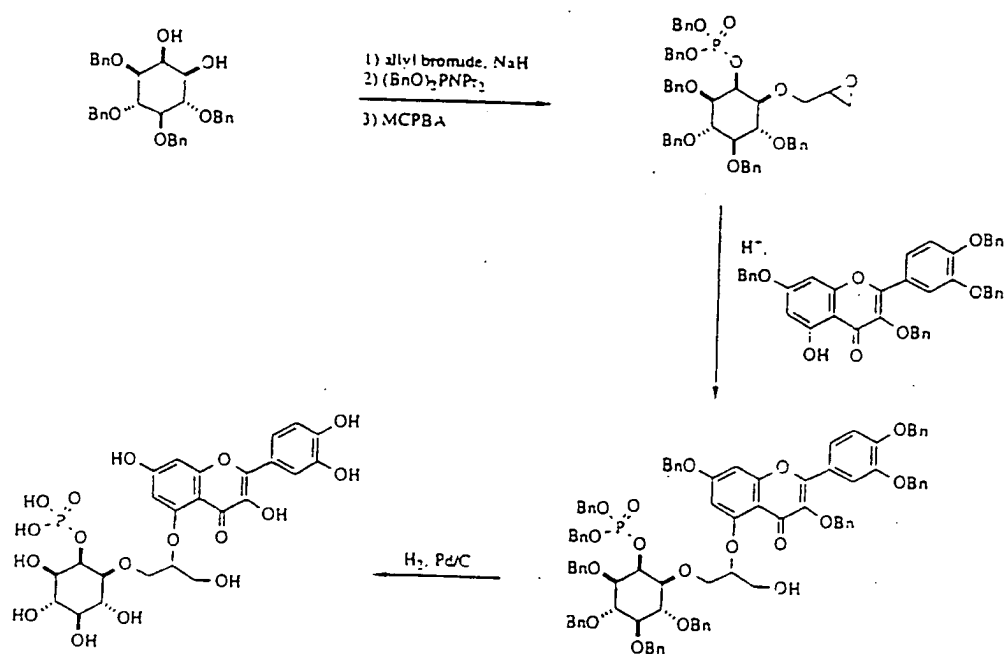


FIGURE 9

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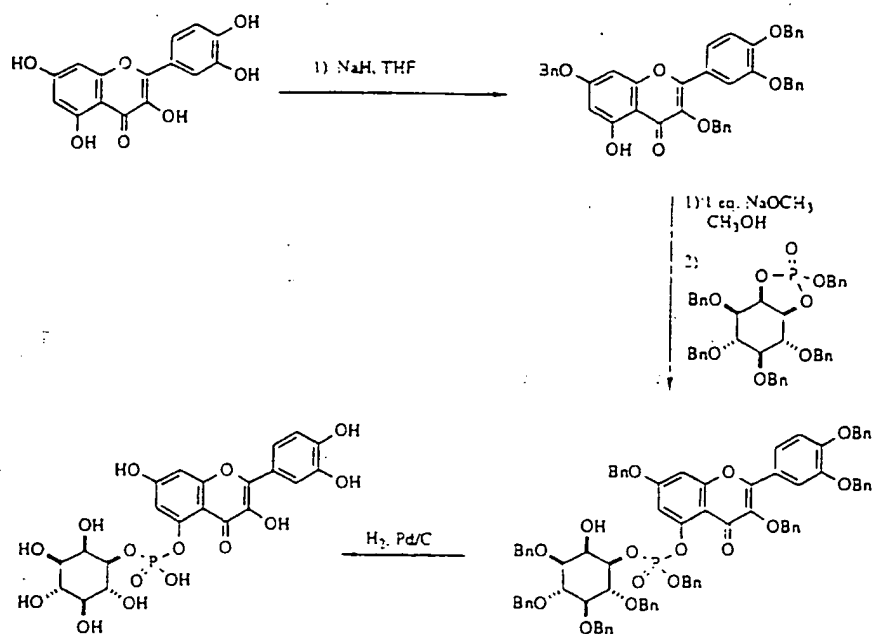
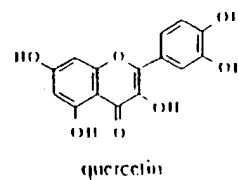
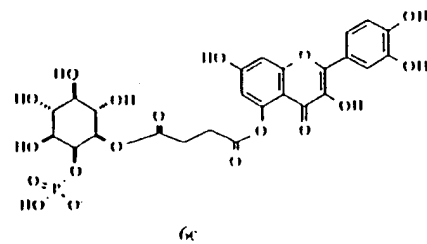
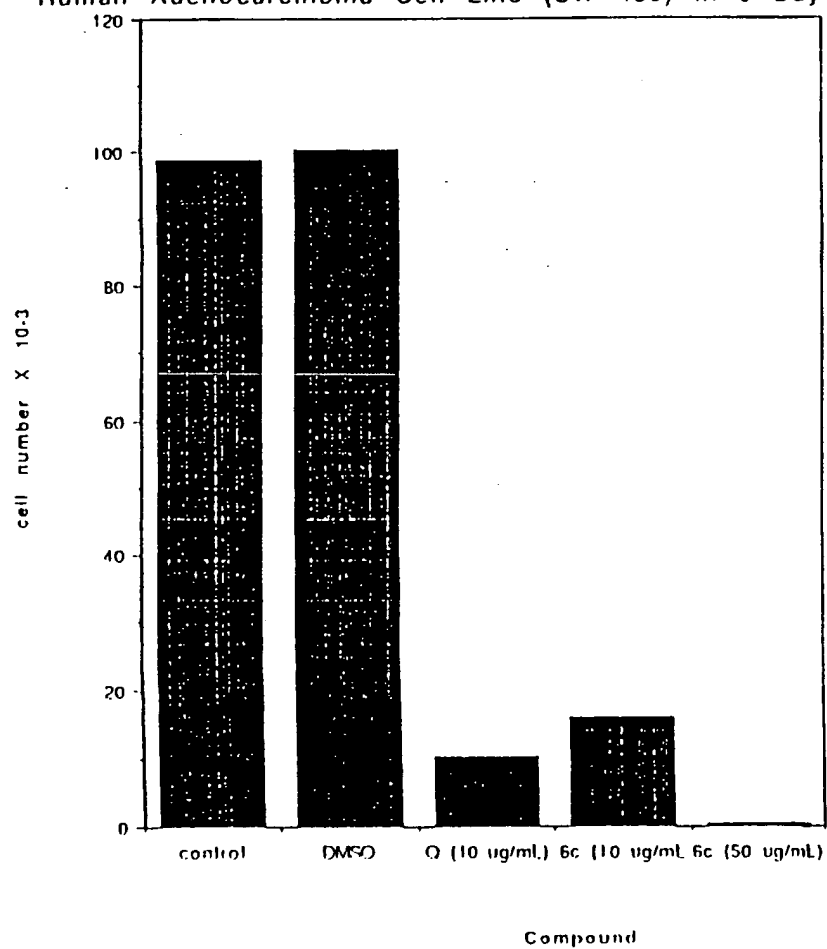


FIGURE 10

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Figure 11

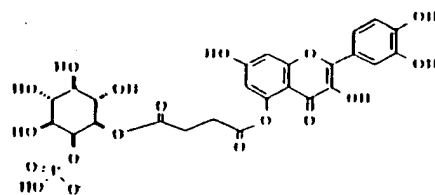
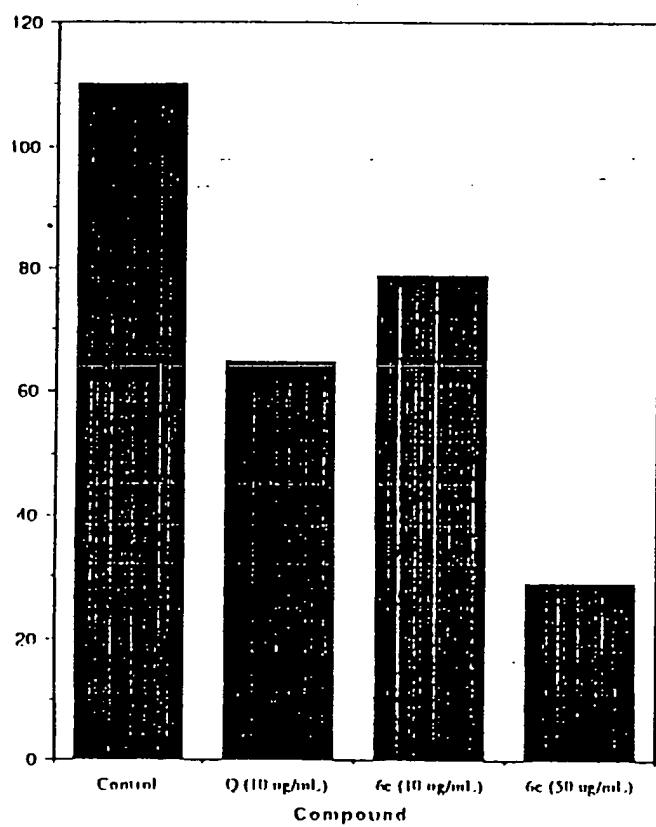
Effect of Quercetin and Inositol-Quercetin Conjugate 6c on a Primary Human Adenocarcinoma Cell Line (SW 480) in 9 Day Culture



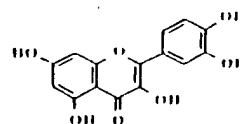
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Figure 12

Effect of Quercetin and Conjugate 6c on a Primary Osteosarcoma Cell Line (MG-63) in 9 Day Culture



6c



quercetin

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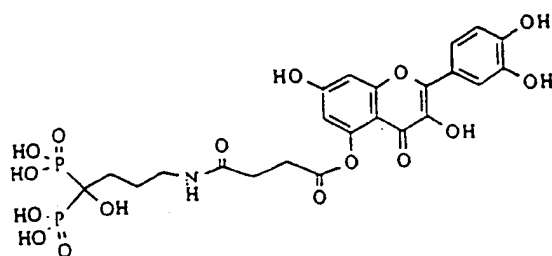


FIGURE 13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/16161

A. CLASSIFICATION OF SUBJECT MATTER IPC(6) : Please See Extra Sheet. US CL : 549/220, 222, 403; 514/456; 536/8; 558/104, 70; 568/833 According to International Patent Classification (IPC) or to both national classification and IPC														
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 549/220, 222, 403; 514/456; 536/8; 558/104, 70; 568/833 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched None Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) CAS ONLINE														
C. DOCUMENTS CONSIDERED TO BE RELEVANT														
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.												
Y	Biochemical and Biophysical Research Communications, Volume 149, No. 3, issued 1987, Saltiel et al, "Inositol glycan mimics the action of insulin on glucose utilization in rat adipocytes..", pages 1084-1092, see especially pages 1084, 1091.	6												
Y	Principles of Medicinal Chemistry, issued 1995, Foye et al, "Anticoagulants, coagulants, and plasma extenders " pages 388-392. See entire document.	6												
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.														
<table border="0"> <tr> <td>* Special categories of cited documents:</td> <td>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>"A" document defining the general state of the art which is not considered to be of particular relevance</td> <td>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>"E" earlier document published on or after the international filing date</td> <td>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>"G" document member of the same patent family</td> </tr> <tr> <td>"O" document referring to an oral disclosure, use, exhibition or other means</td> <td></td> </tr> <tr> <td>"P" document published prior to the international filing date but later than the priority date claimed</td> <td></td> </tr> </table>			* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"G" document member of the same patent family	"O" document referring to an oral disclosure, use, exhibition or other means		"P" document published prior to the international filing date but later than the priority date claimed	
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"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone													
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art													
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"G" document member of the same patent family													
"O" document referring to an oral disclosure, use, exhibition or other means														
"P" document published prior to the international filing date but later than the priority date claimed														
Date of the actual completion of the international search 28 MARCH 1996		Date of mailing of the international search report 22 APR 1996												
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230		Authorized officer ANTHONY BOTTINO Telephone No. (703) 308-1235												

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/16161

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-6,10-25

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/16161

A: CLASSIFICATION OF SUBJECT MATTER: IPC (6):

A61K 31/355; C07C 35/14, 69/76; C07D 311/04; C07F 9/02, 9/28; C07H 15/00

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING This ISA found multiple inventions as follows:

RESTRICTION REQUIREMENT

Since this is a national stage application, the unity of invention standard of PCT Rule 13 and 37 CFR 1.475 was applied in this case.

I. Restriction to one of the following inventions is required:

I. Claims 1-6,10-25 wherein the Chemical Moiety is Inositol-Phosphate, a polyol, and isomers.

II. Claims 1-2,8-18,26-32 wherein the Chemical Moiety is a bisphosphonate and it's isomers.

III. Claims 1-2,6-7,10-18 wherein the Chemical Moiety is a molecule containing a sulfate group and it's isomers.

IV. Claims 1-2,6-7,10-18 wherein the Chemical Moiety is a molecule containing a carboxyl group and it's isomers.

V. Claims 1-2,6-7,10-18 wherein the Chemical Moiety is a molecule containing a carbamate group and it's isomers.

VI. Claims 1-2,6-7,10-18 wherein the Chemical Moiety is a molecule containing an amino acid, polyamine, amino alkyl, peptide or protein.

VII. Claims 1-2,6-7,10-18 wherein the Chemical Moiety is a molecule containing glutathione, glyceraldehyde or derivatives.

VIII. Claims 1-2,6-7,10-18 wherein the Chemical Moiety is a molecule containing a nucleoside, a nucleotide and it's isomers.

The inventions are distinct, each from the other because of the following reasons:

Unity of invention exists only with certain categories of invention as set forth in PCT Rule 13. Note that compounds, corresponding compositions, and method of use and a process of making are considered to form a single, inventive concept as required by PCT Rule 13.1, 37 CFR 1.475 (d). Each group as outlined above are not so linked as they would require separate searches in the prior art and would be expected to raise different issues of novelty or obviousness due to different chemical structure. Further, compounds of Groups I - VIII together do not belong to a "recognized class of chemical compounds in the art" since there is no expectation in the art that members of all eight groups behave in the same way in the context of the claimed invention's pharmacological activity, which is inherently unpredictable.

In view of lack of unity of invention, the requirement for restriction for examination purposes indicated is proper.